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THE ULCEROGENIC EFFECT OF BILE AND BILE ACID IN RATS  
DURING IMMOBILIZATION STRESS

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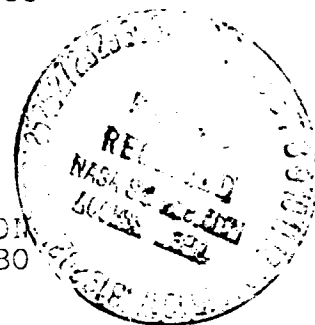
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# THE ULCEROGENIC EFFECT OF BILE AND BILE ACID IN RATS DURING IMMOBILIZATION STRESS

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The duodenal reflux has been discussed for some time as a cause in /539\* the pathogenesis of the gastric ulcer. The reflux of alkaline duodenal juice may destroy the gastric mucosal barrier [15] , so that the acid may directly affect the mucosa. When examining the juices of the empty stomach, high concentrations of bile and/or bile acids were found 33 .

In the present study the effect of oxen bile and bile acid on the gastric mucosa is to be studied under immobilization stress conditions.

## Method

Male Wistar rats (AFH Hanover, Institute of Dr. Hageman) weighing 200-240 g. served as trial animals. The rats were held in plastic boxes (55 x 35 x 20 cm. in size) in groups of 12 animals before trials were made. They were fed Altromin solid fodder and water ad libitum. Immobilization was carried out in cylindrical wire cages 16 cm. long and 5.5 cm. base diameter.

Pretrials with groups of 10 or 12 animals for determination of an approx. 50% ulcer frequency resulted in rats, which had not previously fasted, 30% after 8 hours, 33% after 12 hours, 50% after 16 hours and 75% after 24 hours erosive or ulcerogenic mucosal lesions. An ulcer frequency of 42% resulted after a 4 hour immobilization of the animal when they had not been fed for 24 hours but had received sufficient water.

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\* Numbers in the margin indicate pagination in the foreign text.

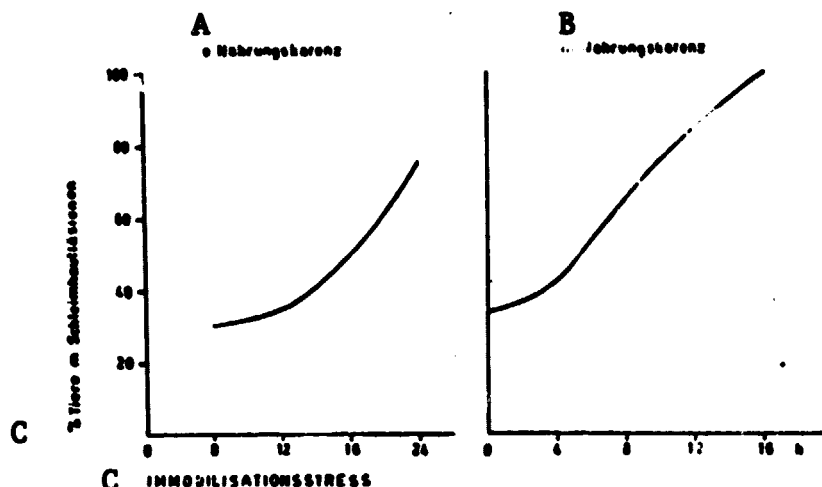


Figure 1: Frequency of Ulcers After Several Hours of Immobilization Stress With and Without Feeding

### Key

A	With Feeding	C	% of Animals with Mucosal Lesions
B	Without Feeding	D	Immobilization Stress

In further pretrials with 50% and 10% oxen bile, with 3.3% crystalline cholic acid and physiological salt solution after a 4 hour immobilization stress, 90% of the animals receiving 10% oxen bile demonstrated mucosal lesions. In the group receiving 50% oxen bile in the stomach, a 50% frequency of ulcers resulted with a high degree of dilated and atonic stomachs. In the case of the animals receiving 3.3% crystalline cholic acid, 30% mucosal lesions resulted; in the control group, receiving physiological salt solution, 20% of the animals demonstrated mucosal lesions.

Defined trial conditions were therefore employing rats weighing 200-240 g., giving the animals no food, but sufficient water, for a 24 hour period and carrying out a 4 hour immobilization after application of oxen bile and bile acids via a stomach tube.

The stomach tube was introduced into the stomach; 2 ml. each pre-warmed to 35-37° C of oxen bile, crystalline cholic acid, Na-deoxycholic acid, Na-glycocholic acid, Na-taurocholic acid and lithocholic acid

were applied at a concentration of 1%, 5%, and 10% in a physiological salt solution. A further group of rats received 2 ml. of physiological salt solution, another received nothing.

### Evaluation

After 4 hours of immobilization the animals were anesthetized by means of ether and killed with the application of a pneumothorax. The stomach was separated 5 mm. below the pylorus, filled with 2 ml. water and fixed for 3-5 minutes in a 4% Formalin solution. The stomachs were subsequently cut open carefully along the large curvature without damaging mucosa and spread on a cork plate. The preparations were immediately examined with the naked eye and with the microscope. Localization and size of mucosal lesions were registered. Only those mucosal lesions microscopically visible were evaluated. A group comparison was made of the animals with gastric mucosal lesions and without these findings. No determination of an ulcer index was made.

The % test was applied, where necessary, for checking results.

### Results

The mucosal lesions were found almost exclusively in the glandular stomach - in only one case an ulcer having the dimensions of a large-sized pinhead was found in the stomach. The lesions were evenly distributed on the front and back wall. The antrum was seldom affected, the duodenum was never affected. The lesions were usually found in the folds, having a deep, wide hemorrhagic base. Microscopically only the mucosa were affected, never the lamina musculavis mucosa.

In addition to the alterations in mucosa already described, in the case of the rats receiving 50% oxen bile and in those receiving 10% Na-glycocholic acid, an extreme dilation of the stomach occurred. In addition, all animals of the 2 groups mentioned were affected by diarrhea.

Frequency of acute mucosal lesions in the glandular stomach of rats where oxen bile and various bile acids were applied in differing concentrations is shown in Table 1.

Table 1: Frequency of Mucosal Lesions in the Glandular Stomach of Rats Where Oxen Bile and Varying Bile Acids Were Applied in Differing Concentrations.

/541

A	B	C		D		E		I	J
		o. 1:1:Cl (0.9 %ig)	pH	n	Ulcerationen pro Gruppe G	Errosionen pro Gruppe H	stat. Signif.		
Gruppe	Substrat								
A <sub>1</sub>	K Rinderbile	1	6.4	12	3	1	—	3	—
A <sub>2</sub>	K Rinderbile	5	6.6	12	5	5	—	6	—
A <sub>3</sub>	K Rinderbile	12	6.6	12	7	16	—	4	+
B <sub>1</sub>	L Cholelsäure	1	4.6	12	1	3	—	9	—
B <sub>2</sub>	L Cholelsäure	5	4.6	12	—	8	—	9	—
B <sub>3</sub>	L Cholelsäure	12	4.7	12	2	12	—	6	—
C <sub>1</sub>	M Na-Deoxycholsäure	1	7.7	12	1	3	—	9	—
C <sub>2</sub>	M Na-Deoxycholsäure	5	9.0	12	1	—	—	11	—
C <sub>3</sub>	M Na-Deoxycholsäure	10	8.3	12	1	—	—	11	—
D <sub>1</sub>	N G-kochohlsäure	1	2.3	12	—	2	—	12	—
D <sub>2</sub>	N G-kochohlsäure	5	1.8	12	—	—	—	12	—
D <sub>3</sub>	N G-kochohlsäure	10	1.8	12	10	4	—	1	+
E <sub>1</sub>	O Na-Taurocholsäure	1	6.3	12	—	—	—	12	—
E <sub>2</sub>	O Na-Taurocholsäure	5	7.6	11	—	4	—	9	—
E <sub>3</sub>	O Na-Taurocholsäure	10	8.1	11	—	3	—	7	—
F <sub>1</sub>	P Lithocholsäure	1	6.6	12	—	2	—	11	—
F <sub>2</sub>	P Lithocholsäure	5	5.9	12	—	4	—	9	—
F <sub>3</sub>	P Lithocholsäure	12	5.8	12	—	15	—	8	—
G	Q NaCl	0.9	6.4	12	—	12	—	7	—
O	—	—	—	20	6	2	—	15	—

\*) bei 5%iger Irrtumswahrscheinlichkeit gegenüber der Kontrollgruppe.

# Key

A	Group	I	No Finding	P	Lithocholic Acid
B	Substrate	J	Statistically Significant	Q	NaCl
C	Soluble in % NaCl (0.9%)	K	Oxen Bile	R	* at 5% Error Probability Compared to Control Group
D	Ulcerations	L	Crystalline Cholic Acid		
E	Erosions	M	Na-Decychoholic Acid		
F	Per Group	N	Na-Glycocholic Acid		
G	Point-Shaped	O	Na-Taurocholic Acid		
H	Band-Shaped				

ORIGINAL MANUSCRIPT  
POOR QUALITY

In only 2 of 246 animals there were 3 ulcers, in 4 other animals /542 there were 2 ulcers per stomach.

Unusual mucosal lesions in the form of wide, deep mucosal bleeding, especially along the limit fold, originated by the application of 10% Na-glycocholic acid. Because of lesion intensity these were rated as ulcerations.

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OF POOR QUALITY

Figure 2: Band-Shaped Bleeding Along the Limit Fold in the Glandular Stomach of the Rat. Condition After the Application of 10% Na-glycocholic Acid and After 4 Hours of Immobilization Stress.

An increase in frequency of ulcers was only proven in the groups D<sub>3</sub>, A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>. A statistically based difference, however, occurred in the  $\chi^2$  test only between the 0 groups and groups A<sub>3</sub> and D<sub>3</sub>.

### Discussion

The present studies of rat stomachs in the case of direct application of bile and bile acids in connection with a 4 hour immobilization stress should serve for examining the effect of the duodenal reflux in connection with the pathogenesis of the gastric ulcer.

The combination of 2 methods for generating peptic mucosal lesions in the animal experiment, in the present studies the combination of immobilization stress with the application of bile and bile acids in varying concentrations, permits more precise statements on the effect of certain materials on the gastric mucosa compared to published



experiments.

With respect to the complex composition of bile, in addition to oxen bile the following bile acids in 1%, 5%, and 10% concentrations were applied in each case: cholic acid, sodium deoxycholic acid, sodium glycocholic acid, sodium taurocholic acid and lithocholic acid. /543

Immobilization stress of rats has been tested for a long period of time as an animal experimental model for generation of gastric mucosal lesions and has been described in various variants ([3] - [8], [22], [24], and others). Several discussions of the pathogenetic course of these stress models have been published in connection with other experiments ([6], [17] and others).

The observation that bile does not cause any phlogistic changes in gastric mucosa was made in the case of transplantation of gastric mucosa to the gallbladder [9]. Instead a cholecystitis was determined in all trial animals.

On the other hand, an increase in mucosal lesions was determined due to a chronic gastritis and attributed to the duodenal reflux of bile [15]. Watkinson [32] already found bile often in his examinations of digestive juice content of empty stomachs in patients with gastric ulcers. A connection between duodenal-pancreatic reflux of bile and gastritis has also been mentioned by other authors [15]. In 1956 Siurala and Tawest [31] described so-called "Gmelin-positive agents" in 46% of extreme cases of atrophy of gastric mucosa and in 31% cases of less severe mucosal atrophy. Capper et al. [10] were able to prove an increased duodenal reflux in the case of gastric ulcers using X-ray methods, Rhodes [30] discovered significantly higher bile acid concentrations in patients with stomach ulcers as opposed to healthy patients. Due to lipophilic and hydrophilic characteristics bile and bile acids are attributed the capability of destroying the gastric mucosal barrier. Ball et al. [2] found a loss in viscosity of gastric mucosal barrier where digestive juices were neutralized. Due to reduced resistance of mucosal barrier the  $H^+$  ions directly affect

the mucosa, in which  $\text{Na}^+$  ions are released from the mucosal cells ([12] , [13] , [23] ). Ivy [23] discovered only 15 minutes after application of bile or bile salts destruction of the functional mucosal barrier with alterations of the described ion flow, Werther [33] observed this result after 30 minutes. Via rediffused  $\text{H}^+$  ions into the mucosa an increased HCl and pepsin secretion results in the mucosa [29] . Lawson [26] determined a chronic gastritis in the case of duodenal reflux especially in connection with bile and pancreatic juice when experimenting with dogs. In the case of experiments with monkeys all degrees of a reflux-esophagitis was generated, as well as an esophageal ulcer after cholecystic ingestion [18] .

The present studies with oxen bile in varying concentrations (1%, 5% and 10%) as well as varying bile acids resulted only in the animals given the 10% oxen bile (group A<sub>3</sub>) and given 10% sodium glycocholic acid (D<sub>3</sub>) a statistically significant increase in frequency of mucosal lesions in the stomach as compared to the control group (Table 2).

The important morphologically and statistically significant alterations in the animal group (D<sub>3</sub>) given 10% sodium glycocholic acid was striking.

The animals demonstrated as a whole a large degree of atony with a considerable dilation of the stomach as well as extended band-shaped areas of mucosal bleeding. The high degree of stomach atony, in connection with a stasis of the stomach as described by Dragstedt must be considered as the reasons for an increased vulnerability of mucosa. To what degree the sodium glycocholic acid has a toxic effect - Cross and Wangenstein reported in 1951 on 8 cats with necrosis of the esophagus wall after perfusion with sodium taurocholic acid and sodium glycocholic acid [1] - remains to be examined.

**Table 2: Summary of the Individual Main Groups Designated by a Letter and Composed of 3 Sub-groups in Each Case and a Comparison of the Animals with Ulcers or Erosions (in this case pre-ulcerations) and Those Where None Were Found. An increased lesion frequency results only in the groups A and D, statistically significant only in A<sub>3</sub> and D<sub>3</sub>.**

	A	B	C	D	E
Gruppe	ulc.	prae. ulc.	u. B.	Zahl d. Tiere	
A	12	9	15	36	
B	2	10	24	36	
C	3	2	31	36	
D	10	3	23	36	
E	—	6	28	34	
F	—	8	28	36	
G	—	5	7	12	
O	4	1	15	20	

**Key**

A Group                      C Pre-ulcerations                      E # of Animals  
 B Ulcer                        D Without Findings

Sodium taurocholic acid, however, showed no visible effect in our experiments compared to the control group.

Except for the important and probably toxic effects of Na-glycocholic acid in a 10% solution only the animal group given oxen bile in a 10% solution showed a statistically significant ulcer frequency compared to the control group. As far as the oxen bile is concerned, the results are in agreement with those published, especially with clinical observations. With the exception of the 10% Na-glycocholic acid, however, the same statement may not be made about the individual bile acids tested, or their natrium salts. The effect of bile in the pathogenesis of ulcerous gastric mucosal lesions may be considered pioneering, taking the experimental results into consideration and is especially apparent in combination with other ulcerogenic factors, represented in the present experiments by immobilization stress.

## Summary

The present work studies the effect of oxen bile and individual bile acids, or their natrium salts in differing concentrations on the gastric mucosa of rats in combination with immobilization stress. A statistically significant higher frequency of ulcers was only determined in the application of 10% oxen bile. Dosages of 10% sodium glycocholic acid demonstrated strong toxic damage with atonic dilation of the stomach and extensive mucosal bleeding.

The results gained are explained in connection with published clinical and animal experiments.

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